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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07D 501/34, A61K 31/545 C07C 87/28	A1	**(11) International Publication Number:** WO 87/ 03876 **(43) International Publication Date:** 2 July 1987 (02.07.87)

(21) International Application Number: PCT/GB86/00790

(22) International Filing Date: 22 December 1986 (22.12.86)

(31) Priority Application Number: 8531609

(32) Priority Date: 23 December 1985 (23.12.85)

(33) Priority Country: GB

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(81) Designated States: BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), NL (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BENZATHINE CEPHALOTHIN, A PROCESS FOR ITS PREPARATION AND COMPOSITIONS CONTAINING IT

(57) Abstract

Benzathine cephalothin for use in the treatment of bacterial infection in animals, in particular for the treatment or prophylaxis of mammary disorders and keratoconjunctivitis.

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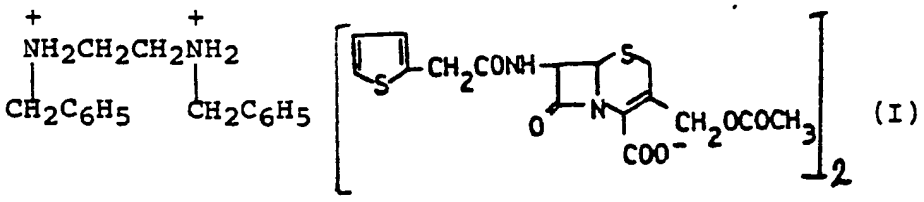
Benzathine cephalothin, a process for its preparation and compositions containing it.

The present invention relates to a novel β lactam compound and the process for its production and to pharmaceutical composition containing the compound.

U.K. patent 982252 discloses heterocyclic substituted acyl derivatives of cephalosporin C including 7-(2-thienylacetamido)cephalosporanic acid, known as cephalothin. This compound, particularly as its sodium salt, has become widely used for human patients as an antibacterial agent having potent activity against a broad spectrum of gram positive and gram negative bacteria. One disadvantage of cephalothin as an antibiotic is that it is an irritant when used intramuscularly.

We have now found a novel salt of cephalothin which has particular utility in the treatment of bacterial infection in animals.

According to the present invention there is provided a compound of formula I:



(I)

Hereinafter the compound of formula I will be referred to by its trivial name benzathine cephalothin.

The major utility of benzathine cephalothin is as an antibacterial . It has been found however to be particularly effective in the treatment of mammary disorders, particularly in the treatment of bovine mastitis in dry cows. The compound has also been found to be surprisingly effective in the treatment of keratoconjunctivitis, and in particular bovine keratoconjunctivitis, which is a highly contageous disease of cattle caused by Moraxella bovis.

Thus the present invention also provides a veterinary composition which comprises benzathine cephalothin and a veterinarily-acceptable carrier.

The compositions suitably comprise a suspension of benzathine cephalothin in an aqueous medium or more preferably in a non-toxic veterinarily-acceptable oil. When an oily vehicle is employed it may comprise a mineral oil or a vegetable oil such as arachis oil, sesame oil, corn oil, cottonseed oil, soyabean oil, olive oil or a fractionated coconut oil. A preferred vehicle is the fractionated coconut oil described in German OS 2635476. Suitable commercially available oils are Miglylol (Trade Mark) or Neobee (Trade Mark).

In compositions in accordance with this aspect of the invention the active ingredient will normally represent 0.1 to 40%, more suitably 1 to 40% w/w. Particularly suitable ranges are 5 to 30% w/w.

When the compound of the invention is used in the treatment or prophylaxis of bovine mastitis in dry cows it is normally provided in an oily formulation for intramammary use. Since slow release is required for the therapy of dry cows a hydrophobic oily vehicle which has been strongly gelled with a gelling agent such as aluminium stearate may be used. Thickening agents such as 12-hydroxystearin, beeswax, hydrogenated peanut or castor oil or soft or hard paraffin may be used.

The composition may also contain pain relieving agents, corticosteroids and the like. Surfactants such as Tween (Trade Mark), Span (Trade Mark) or Lanette wax may also be present. It may also be desirable to include an antioxidant such as butylated hydroxyanisole (Embanox - Trade Mark) in certain formulations.

Preferably the veterinary compositions for intramammary use are formulated as unit doses containing a therapeutically effective amount of benzathine cephalothin. For use in the dry cow the preferred unit dose may contain 100 to 1000 mg more preferably 250 to 600 mg of benzathine cephalothin. A typical dose is 500 mg.

A single dose of the composition will normally contain 1 to 20g of the formulated composition, preferably 2 to 10g. Typical formulations may contain 3g or 8g.

In the treatment of mastitis in dry cows a single dose applied to the infected quarter may be effective. However one or more further doses may be applied depending on the length of the dry period.

The formulation would suitably be administered to the cow by means of an intramammary syringe, a tube or by other suitable packs which contain a unit dose of the formulation. Such a syringe is provided with a cannula nozzle for insertion into the teat to allow extrusion of the formulation directly into the mammary gland via the streak canal.

Thus the present invention also provides a method of treatment or prophylaxis of mammary disorders in animals which method comprises the intramammary administration of an effective amount of a composition of benzathine cephalothin and an aqueous or oily vehicle.

Suitably compositions of the present invention for the treatment of kevatoconjunctivitis contain benzathine cephalothin in an oily vehicle.

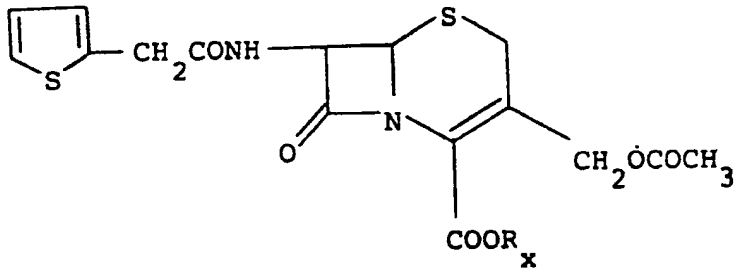
Suitably the oily vehicle is a mineral oil base, preferably liquid paraffin containing 0 to 5% by weight of composition of aluminium stearate and from 0 to 2% by weight of composition of stearic acid. Alternatively the oily vehicle may comprise a vegetable oil such as arachis oil or a fractionated coconut oil such as Miglylol or Neobee.

The dosage regime will vary with the size of the sufferer. A suitable dose unit is typically between 20 and 200 mg of benzathine cephalothin. For the treatment of cattle a dose of about 150 mg of benzathine cephalothin is appropriate but a smaller dose, for example 50 mg may be sufficient for the treatment of keratoconjunctivitis in domestic animals such as cats and dogs. Frequently a single application

of the formulation will be sufficient. However severe infections may require more than one application.

Thus the present invention further provides a method of treatment of keratoconjunctivitis in animals which comprises administering to the animal by topical instillation an effective amount of a composition of benzathine cephalothin and a veterinarily acceptable oil as carrier. A number of suitable containers for instillation of a formulation onto an infected eye are in common use. Preferably the container is a sealed aluminium tube or a polyethylene syringe.

The present invention also provides a process for the preparation of benzathine cephalothin which process comprises reacting solution of a compound of the formula (II).



(II)

wherein R^X is hydrogen or a carboxy protecting group, with a solution of N,N-dibenzylethylenediamine or a salt thereof. The reactants may be dissolved in any suitable aqueous or non-aqueous solvent. Particularly high yields are obtained by reacting cephalothin (free acid) with N,N-dibenzylethylenediamine (free base) in a non-aqueous solvent. A preferred solvent is acetone or methanol.

Veterinary compositions of the present invention may be prepared by mixing benzathine cephalothin with the vehicle and with any other components of the formulation. The process may suitably be carried out as follows:-

(a) the oil is heated, the gelling or thickening agent is mixed in and the oil allowed to cool,

(b) the powdered active ingredient is mixed into the base with stirring and

(c) high shear mixing equipment is used to produce a fine monogenous dispension.

The formulation is then packed in an appropriate form for administration, eg. syringe or tube.

The following Examples illustrate the invention.

Example 1

Sodium cephalothin (16.8g; 0.04 mole) was dissolved in water (150cm^3), diluted with acetone (150cm^3) and filtered. To the vigorously stirred solution was added a filtered solution of N,N-dibenzylethylenediamine diacetate (7.2g; 0.02 mole) in water (70cm^3). The product precipitated immediately as a white gelatinous solid. The mixture was stirrred for 30 minutes and the precipitate removed by filtration. The filter cake was slurry washed in water (300cm^3)for 1 hour, filtered off and dried at 35^o for 24 hours.

Yield = 17.9g (86.9% of theory)

Analysis

% Benzathine (f.b.) = 23.2 (Theory = 23.3%)
% Cephalothin (f.a.) = 77.8 (Theory = 76.7%)

Example 2

Sodium cephalothin (4.2; 0.01 mole) was dissolved in water (40cm^3) and diluted with methanol (40cm^3). To this stirred solution was added a filtered solution of N,N-dibenzylethylenediamine diacetate (1.8g; 0.005 mole), in water (20cm^3). The salt precipitated out immediately. The mixture was stirred vigorously for 30 minutes and the product removed by filtration. The ''filter cake'' was washed well with water (80cm^3) and dried at 35^o for 24 hours.

Yield = 4.3g (83.3% of theory)

Analysis

% Benzathine (f.b) = 23.8 (Theory = 23.3%)
% Cephalothin (f.a.) = 75.6 (Theory = 76.7%)

Example 3

Sodium cephalothin (4.2g; 0.01 mole) was dissolved in water (40cm^3) and with good stirring added a filtered solution of N,N-dibenzylethylenediamine diacetate (1.8g; 0.005 mole) in water (20cm^3). The salt precipitated immediately and after stirring for 30 minutes was removed by filtration and slurry washed in water (100cm^3), washed with acetone (100cm^3) and dried at 35^o for 24 hours.

Yield = 4.1g (79.5% of theory)

Analysis

% Benzathine (f.b.) = 24.0 (Theory = 23.2%)

% Cephalothin (f.a) = 76.1 (Theory = 76.7%)

Example 4

Sodium cephalothin (84g; 0.2 mole) was dissolved in water(420 cm^3) at ambient temperature and filtered. Benzathine diacetate (36g; 0.1 mole) was dissolved in water (360 cm^3) at ambient temperature, filtered, and slowly added to the vigorously stirred sodium cephalothin solution. When the addition was complete acetone (390 cm^3) was added to facilitate stirring. Stirring was continued for 30 minutes to ensue homogeneity. The product was removed by filtration and washed free of sodium acetate by reslurrying in 50% aqueous acetone. The filter-bed was washed with acetone to aid drying and the product dried in a fan oven at 35° for 48 hours (Note 4).

Yield = 100.5g (97% of theory)

Analysis (Typical)

Cephalothin content by hplc	78.18%
Benzathine content by hplc	24.6%
Acetone	0.1%
Water	0.1%
Acetate	0.3%

The polymorphic form is readily determined by IR spectroscopy.

Example 5

Cephalothin (free acid) (3.05g; 0.007 mole) was dissolved in acetone (50cm^3) and with stirring added to a solution of N,N-dibenzylethylenediamine (free base) (0.84g; 0.0035 mole) in acetone (50cm^3). A gelatinous precipitate immediately appeared which was removed by filtration, after vigorous stirring for 30 minutes, washed well with acetone (50cm^3) and dried at 35° for 24 hours.

Yield = 3.55g (97% of theory)

Example 6

Cephalothin (free acid) (3.05g; 0.007 mole) was dissolved in methanol (50cm^3) and with stirring added a solution of N,N-dibenzylethyleneddiamine (free base) (0.084g; 0.0035 mole) in methanol (50cm^3). The product precipitated immediately and stirring was continued for 30 minutes. The product was filtered off, washed well with methanol and dried at 35° for 24 hours.

Yield = 3.6g (99% Of theory)

Example 7

	g	%
benzathine cephalothin	83.3	16.667
12-hydroxystearin (Thixcin R)	20	4.0
colloidal silica (Aerosil R972)	5	1.0
butylated hydroxyanisole (Embanox)	.1	0.02
arachis oil	to 500	100

The composition was prepared as follows.

20g of Thixcin R 0.1g of Embanox and 5g of colloidal silica were dissolved in dried Arachis oil by heating to above 150°C for one hour and stirring and then allowing to cool. 83.3g of benzathine cephalothin was then incorporated into this thickened base by high shear stirring and the weight adjusted to 500g by the addition of further Arachis oil.

The suspension was filled as 3g doses into intramammary syringes.

Example 8

	g	%
benzathine cephalothin	83.3	16.667
aluminium stearate	15.35	3.069
liquid paraffin	480.61	96.122
stearic acid	4.04	0.808

A gel of the mineral oil base was formed by dissolving the aluminium stearate and stearic acid in the heated liquid paraffin. After cooling the benzathine cephalothin was incorporated by high shear stirring and the weight adjusted to 500g with further liquid paraffin.

The suspension was filled as 8g doses into intramammary syringes.

Example 9

An ointment formulation was aseptically prepared from

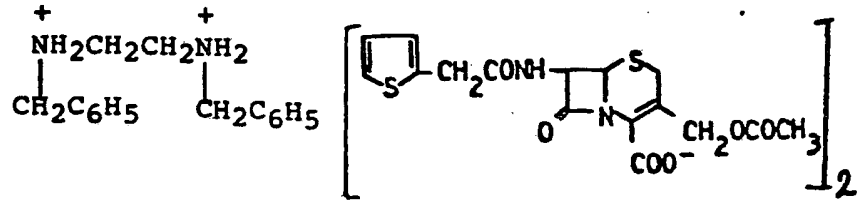
		% w/w of composition
benzathine cephalothin		16.67
liquid paraffin BP	) mineral	80.10
aluminium stearate	) oil	2.56
stearic acid BPC	) base	0.67

The homogenous composition was prepared as described in Example 3.

The suspension was then packaged into 1 ml polyethylene syringes on aluminium tubes. This constitutes a single dose form containing 125mg of benzathine cephalothin suitable for treatment of bovine infectious kerataconjunctivitis.

Claims

1. Benzathine cephalothin which is the compound of formula (I):



(I)

2. A veterinary composition which comprises benzathine cephalothin and a veterinarily acceptable carrier.

3. A veterinary composition as claimed in claim 2, comprising from 0.1 to 40% w/w of benzathine cephalothin.

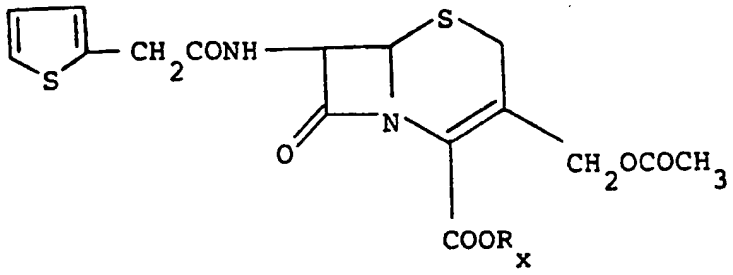
4. A veterinary composition as claimed in claim 2 or claim 3, comprising a suspension of benzathine cephalothin in an aqueous medium or in a non-toxic veterinarily acceptable oil.

5. A veterinary composition as claimed in claim 4, wherein the non-toxic veterinarily acceptable oil is a mineral oil or a vegetable oil.

6. A veterinary composition as claimed in claim 5, wherein the mineral oil is liquid paraffin containing 0 to 5% by weight of composition of aluminium stearate and from 0 to 2% by weight of composition of stearic acid.

7. A veterinary composition as claimed in claim 5, wherein the vegetable oil is arachis oil, sesame oil, corn oil, cottonseed oil, soyabean oil, olive oil or a fractionated coconut oil.

8. A process for the preparation of a compound as claimed in claim 1, which process comprises reacting a solution of a compound of formula (II).



(II)

with a solution of N,N-di-benzylethylenediamine or a salt thereof.

9. A method of treatment or prophylaxis of mammary disorders in animals, which method comprises the intramammary administration of an effective amount of a composition of benzathine cephalothin and an aqueous or oily vehicle.

10. A method of treatment of keratoconjunctivitis in animals, which comprises administering to the animal by topical installation, an effective amount of a composition of benzathine cephalothin and a veterinarily acceptable oil as carrier.

11. The use of benzathine cephalothin for the manufacture of a medicament for the treatment or prophylaxis of mammary disorders in animals.

12. The use of benzathine cephalothin for the manufacture of a medicament for the treatment of keratoconjunctivitis in animals.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 86/00790

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 D 501/34; A 61 K 31/545; C 07 C 87/28

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 07 D 501/00; C 07 C 87/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FR, A, 2424279 (ANTIBIOTICS) 23 November 1979, see the whole document, particularly example 1	1
A	FR, A, 2268526 (LABORATORIO FARMACEUTICO QUIMICO LAFARQUIM S.A.) 21 November 1975, see page 10, claims	1
A	US, A, 3129224 (SMITH KLINE & FRENCH LABORATORIES) 14 April 1964 see column 1, lines 10-56	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th March 1987	29 AVR. 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers xx..... because they relate to subject matter not required to be searched by this Authority, namely:

xx Claims 9-10
See PCT Rule 39.1(iv):
Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 86/00790 (SA 15579)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/04/87

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A- 2424279	23/11/79	DE-A-	2914191	31/10/79
		GB-A-	2019838	07/11/79
		JP-A-	54154783	06/12/79
FR-A- 2268526	21/11/75	BE-A-	827826	31/07/75
		NL-A-	7504747	27/10/75
		DE-A-	2516251	06/11/75
		JP-A-	50142723	17/11/75
US-A- 3129224		None		